Amendments to the Specification

Please amend the specification as indicated below.

Please replace the title on page 1 of the application with the following amended title:

~~CD4-GAMMA2 AND CD4-IgG2 CHIMERAS~~ CELLS EXPRESSING A CD4-IgG2 CHIMERIC HETEROTETRAMER

Please replace the paragraph beginning on page 1, line 3 (as amended in the June 7, 1995 Preliminary Amendment) with the following amended paragraph:

This application is a continuation of U.S. Serial No. 07/960,440, filed December 8, 1992, ~~which is based on~~ now abandoned, a national stage application of PCT International Application No. PCT/US92/01143, filed February 10, 1992, which is a continuation-in-part of U.S. Serial No. 07/653,684, filed February 8, 1991, now abandoned.

Please replace the paragraphs beginning on page 15, line 11 to page 16, line 14 with the following amended paragraphs:

Figure 6: Secretion of CD4-gamma2 chimeric heavy chain homodimer from transfected cells. Cos-M5 cells were mock transfected, transfected with CD4-gamma1 chimeric heavy chain mammalian expression vector DNA, or transfected with CD4-IgG2-pcDNA1. At 48-72 hours post-transfection, the cells were radiolabelled with ^{35}S-methionine. Radiolabelled medium was precipitated with ~~Protein-A sepharose~~ Protein A-Sepharose® beads. The precipitated proteins were analyzed by SDS-PAGE under reducing or non-reducing conditions and were visualized by fluorography. Lane M, medium from mock

transfected cells; Lane 1, medium from cells transfected with CD4-gamma1 chimeric heavy chain mammalian expression vector DNA; Lane 2, medium from cells transfected with CD4-IgG2-pcDNA1 DNA.

Figure 7: Precipitation of HIV-1 gp120 with CD4-gamma2 chimeric heavy chain homodimer. Cos-M5 cells were mock transfected, transfected with CD4-gamma1 chimeric heavy chain mammalian expression vector DNA, or transfected with the CD4-IgG2-pcDNA1. At 48-72 hours post transfection, unlabelled aliquots of medium were incubated with an aliquot of ^{35}S-methionine labelled gp120. The complexes were precipitated with Protein ~~A-sepharose~~ A-Sepharose® beads. The precipitates were then analyzed by SDS-PAGE followed by fluorography. Lane M, medium from mock transfected cells; Lane 1, medium from cells transfected with CD4-gamma1 chimeric heavy chain mammalian expression vector DNA; Lane 2, medium from cells transfected with CD4-IgG2-pcDNA1 DNA.

Figure 8: Purification of CD4-gamma2 chimeric heavy chain homodimer from CHO cell-conditioned medium. Stable CHO cells constitutively secreting CD4-gamma1 chimeric heavy chain homodimer, or CD4-gamma2 chimeric heavy chain homodimer, were grown in roller bottles. Conditioned medium was passed over a Protein ~~A-sepharose~~ A-Sepharose® column and bound material was eluted from the column. The peak fractions were identified by SDS-PAGE followed by silver staining and pooled. The purified proteins were then analyzed by SDS PAGE under reducing conditions followed by silver staining.

Please replace the paragraphs beginning on page 16, line 34 to page 18, line 5 with the following amended paragraphs:

Figure 11: Purification of CD4-gamma2 chimeric heavy chain

homodimer. Stable CHO cells constitutively secreting CD4-gamma2 chimeric heavy chain homodimer were grown in roller bottles. Conditioned medium was passed over a Protein ~~A-sepharose~~ A-Sepharose® column and bound material was eluted from the column (see Figure 8). The peak fractions were then pooled and passed over an ~~S-sepharose~~ S-Sepharose® column. After extensive washes, the CD4-gamma2 chimeric heavy chain homodimer was eluted with 50mM BES pH 7.0, 500mM NaCl. The peak fractions were identified by SDS-PAGE followed by silver staining, pooled, and concentrated. The pooled, concentrated CD4-gamma2 chimeric heavy chain homodimer was then applied to a ~~Sephacryl~~ Sephacryl® S-300HR column preequilibrated and run with PBS.

The peak fraction corresponding to purified CD4-gamma2 chimeric heavy chain homodimer was identified by SDS-PAGE followed by silver staining. The peak fractions were then pooled and concentrated. The purified protein was then analyzed by SDS-PAGE under non-reducing and reducing conditions followed by silver staining. Lane 1: approximately 1.5 µg protein run under non-reducing conditions, Lane 2: approximately 1.5 µg protein run under reducing conditions.

Figure 12: Secretion of CD4-IgG2 chimeric heterotetramer from stably transfected cells. CHO cells stably expressing both CD4-IgG2 chimeric heavy chains and CD4-kappa chimeric light chains were radiolabelled with ^{35}S-methionine and cysteine. Radiolabelled medium was precipitated with ~~Protein-A sepharose~~ Protein A-Sepharose® beads. (A) The precipitated proteins were analyzed by SDS-PAGE under non-reducing conditions, and were visualized by fluorography. Lane 1: medium from untransfected CHO cells, Lane 2: medium from cells stably expressing both the CD4-IgG2 chimeric heavy chains, and CD4-kappa chimeric light chains. (B) An

identical sample to that run in lane 2 from (A) was run on SDS-PAGE under nonreducing conditions. The lane from this SDS-PAGE gel was excised and the proteins reduced by incubation of the gel slice for 45 minutes at 4°C in equilibration buffer (62.5mM TrisHC1 pH 6.8, 2.3% SDS, 5% β-mercaptoethanol, 10% glycerol). After incubation of the gel slice under reducing conditions, the proteins contained within the gel were analyzed by SDS-PAGE and visualized by fluorography.

Please replace the paragraph beginning on page 32, line 8 to page 33, line 7 with the following amended paragraph:

The human CD4 cDNA was excised from the plasmid pSP6T4 (4) as an EcoR1/Stul restriction fragment. The 0.70 kilobase fragment was isolated and cloned into EcoR1/Smal digested M13mp18. This intermediate vector (M13mp18(CD4)) was then isolated, linearized with Pstl, purified, and treated with Bacterial Alkaline Phosphatase (BAP). The 2.0 Kb Pstl/Pstl fragment from the plasmid pBr gamma2 containing the human gamma2 heavy chain gene (36) (containing the hinge, CH2, and CH3 exons) was isolated and cloned into the BAP-treated M13mp18/CD4 vector. Resulting recombinants were then screened for the correct orientation of the Pstl fragment (with respect to the CD4 sequence) to obtain a vector which contains in tandem CD4(EcoR1/Stul) - gamma2(Pstl/Pstl). To obtain a CD4-gamma2 chimeric heavy chain gene, oligonucleotide-mediated site-directed mutagenesis was performed to juxtapose the CD4 and gamma2 heavy chain DNA sequences, ligating the CD4 sequence in frame to the hinge exon. The resulting chimeric DNA molecule encodes a protein containing the V1V2 domains of CD4 followed by the hinge, CH2, and CH3 domains of gamma2 heavy chain (Figure 1A). Mutagenesis was performed on single-stranded DNA isolated from recombinant phage from transformed TG1 cells

(Amersham). Briefly, template DNA was annealed with a 34-mer oligonucleotide (5'-GACACAACATTTGCGCTCGAAAGCTAGCACCACG-3'), containing sequences which join the last codon encoding Phe(179) from V1V2 of CD4 to the first codon of the hinge for IgG2 (encoding Glu) (Figures 1A and 3). After second strand synthesis, double stranded DNA was transformed into competent TG1 cells. Isolated plaques were then grown in fresh TG1 cells and single stranded DNA was purified for DNA sequencing. All mutations were verified and confirmed by dideoxy sequencing using the ~~Sequenase~~ Sequenase® system (USB). Plaques containing the chimeric gene with the correct sequence were then grown in TG1 cells, and Rf DNA (designated CD4-IgG2-Rf) was isolated from the cells.

Please replace the paragraph beginning on page 34, lines 3-16 with the following amended paragraph:

CosM5 cells grown in DMEM containing 10% fetal calf serum are split to 75% confluence. On the following day, the cells are transfected for 16-20 hours with 5 micrograms of CsCl-purified CD4-IgG2HC-pRcCMV DNA and 5 micrograms of CsCl-purified CD4-kLC-pRcCMV plasmid DNA by the standard ~~CaPO(4)~~ $CaPO_4$ precipitation technique. After transfection, fresh medium is added to the cells. Analysis of the products synthesized 48-72 hours post-transfection is performed by radiolabelling of transfectants with ^{35}S-methionine for 12-18 hours followed by precipitation of media and cell lysates using anti-CD4 antibodies or by incubation with Protein ~~A-sepharose~~ A-Sepharose® beads alone followed by SDS-PAGE under reducing or non-reducing conditions. In addition, analysis of media and cell lysates is performed 48-72 hours post-transfection by standard Western blotting procedures.

Please replace the paragraphs beginning on page 35, line 6 to page 36, line 5 with the following amended paragraphs:

CD4-gamma2 chimeric heavy chain homodimer was purified in a single step using Protein ~~A-Sepharose~~ A-Sepharose® column chromatography. CHO cells secreting CD4-gamma2 chimeric heavy chain homodimer were grown to high density in roller bottles in medium containing alpha MEM with 10% IgG-free fetal calf serum. Conditioned media was collected, clarified by centrifugation, and diluted 1:1 with PBS with/or without detergent (i.e. ~~Tween~~ Tween®) in this and subsequent buffers. The diluted media was then applied to a 5ml column of Protein ~~A-Sepharose~~ A-Sepharose® fast flow previously equilibrated with PBS, at a flow rate of 60ml/hour. After extensive washing, the specifically bound material was eluted with 100mM glycine/HCl, pH 3.5, directly into an aliquot of 1M Tris.HCl pH 8.0 to immediately neutralize the eluted fractions. The fractions were then analyzed by SDS-PAGE under reducing and non-reducing conditions followed by silver staining and pooled (Figure 8).

The pooled fractions were then applied to a 10 ml column of ~~S-sepharose~~ S-Sepharose® fast flow previously equilibrated with 50mM BES pH 7.0 at a flow rate of 120ml/hr. After application of sample, a step elution gradient (consisting of the following 4 steps: 5 column volumes of 50mM BES pH 7.0, 4 column volumes of 50mM BES pH 7.0, 100mM NaCl, 6 column volumes of 50mM BES pH 7.0, 225mM NaCl, followed by 8 column volumes of 50mM BES pH 7.0, 500mM NaCl) was employed for specific elution of the CD4-gamma2 chimeric heavy chain homodimer. The CD4-gamma2 chimeric heavy chain homodimer was eluted from the column in 50mM BES pH 7.0, 500mM NaCl. The peak fractions were then pooled and concentrated to ~~yeild~~ yield a final protein concentration

of at least 1 mg/ml. The pooled and concentrated fractions were then applied to a 120 ml column of ~~Sephacryl~~ Sephacryl® S-300HR previously equilibrated with PBS, at a flow rate of 8ml/hr. The CD4-gamma2 chimeric heavy chain homodimer fraction was specifically eluted in PBS, and concentrated to at least lmg/ml.

Please replace the paragraph beginning on page 36, lines 10-23 with the following amended paragraph:

CosM5 transfectants expressing CD4-gamma2 chimeric heavy chain homodimer were incubated for 72 hours in DMEM containing 10% IgG-free fetal calf serum. Unlabelled medium was then collected and used to precipitate ^{35}S-methionine-radiolabelled HIV gpl20. After incubation of CD4-gamma2 chimeric heavy chain homodimer containing medium containing ^{35}S-methionine-labelled gp120, the complexes were adsorbed to Protein ~~A-sepharose~~ A-Sepharose®. Protein ~~A-sepharose~~ A-Sepharose® complexes were recovered by centrifugation, and the precipitates were analyzed by SDS-PAGE under reducing conditions followed by fluorography (Figure 7). Alternatively, aliquots of purified CD4-gamma2 chimeric heavy chain homodimer from CHO cells were also used to precipitate ^{35}S-radiolabelled gpl20 using the same procedure.

Please replace the paragraph beginning on page 40, line 30 to page 41, line 24 with the following amended paragraph:

In order to excise a fragment containing the CH1 exon of the human gamma2 heavy chain gene, the plasmid pBr gamma2 (36) is digested with SacII, and the SacII sites are then made flush using T4 DNA polymerase. After heat inactivation of the polymerase, the fragment is then digested with Pst1. The resulting SacII(flush)- Pst1 fragment containing the

CH1 exon is then purified and ligated to the M13mp18(CD4) vector described in the above paragraph. After transformation of competent TG1 cells, the resulting recombinants are screened by restriction analysis for the presence of both CD4 and CH1 sequences which contain in tandem CD4 (EcoR1/Stu1) - CH1 (SacII (flush)/Pst1). Oligonucleotide-mediated site-directed mutagenesis is then performed to juxtapose the CD4 and CH1 sequences in frame. The resulting chimeric DNA molecule contains the V1V2 domains of CD4 fused to the CH1 domain of gamma2 heavy chain. Mutagenesis is performed on single-stranded DNA isolated from recombinant phage from transformed TG1 cells (Amersham). Template DNA is annealed with a 33-mer oligonucleotide (5'-GGGCCCTTGGTGGAGGCGAAAGCTAGCACCACG-3') containing sequences which join the last codon encoding Phe (179) from V1V2 of CD4 to the first codon of the CH1 domain for gamma2 heavy chain (encoding Ala). After second strand synthesis, double stranded DNA is transformed into competent TG1 cells. Isolated plaques are then grown in fresh TG1 cells and single-stranded DNA is purified for DNA sequencing. All mutations are confirmed by dideoxy sequencing using the ~~Sequenase~~ Sequenase® system (USB). Plaques containing the chimeric genes with the correct sequence as determined by restriction analysis are then grown in TG1 cells, and the Rf DNA is isolated from the cells.

Please replace the paragraph beginning on page 45, lines 19-34 with the following amended paragraph:

CD4-IgG2 chimeric heterotetramers are purified using Protein ~~A-Sepharose~~ A-Sepharose® column chromatography. CHO cells secreting CD4 IgG2 chimeric heterotetramers are grown to high density in roller bottles in medium containing alpha MEM with 10% IgG-free fetal calf serum. Conditioned

media is collected, clarified by centrifugation, and diluted 1:1 with PBS with/or without detergent (i.e. ~~Tween~~ Tween®) in this and subsequent buffers. The diluted media is then applied to a 5ml column of Protein ~~A-Sepharose~~ A-Sepharose® fast flow previously equilibrated with PBS, at a flow rate of 60ml/hour. After extensive washing, the bound material is eluted with 100mM glycine/HC1, pH 3.5, directly into an aliquot of 1M Tris.HCl pH 8.0 to immediately neutralize the eluted fractions. Fractions are then analyzed by SDS-PAGE under reducing and non-reducing conditions followed by silver staining and pooled (Figure 8).

Please replace the paragraph beginning on page 46, lines 3-15 with the following amended paragraph:

CosM5 transfectants expressing CD4-IgG2 chimeric heterotetramers are incubated for 72 hours in DMEM containing 10% IgG-free fetal calf serum. Unlabelled medium is then collected and used to precipitate ^{35}S-methionine-radiolabelled HIV gp120. After incubation of CD4-IgG2 chimeric heterotetramer containing medium with ^{35}S-methionine-labelled gp120, the complexes are adsorbed to Protein ~~A-sepharose~~ A-Sepharose®. Protein ~~A-sepharose~~ A-Sepharose® complexes are recovered by centrifugation, and the precipitates are analyzed by SDS-PAGE followed by fluorography. Alternatively, aliquots of purified CD4-IgG2 chimeric heterotetramers from CHO cells are also used to precipitate ^{35}S-radiolabelled gp120 using the same procedure.

Please replace the paragraphs beginning on page 49, line 3 to page 51, line 31 with the following amended paragraphs:

The CD4-gamma2 chimeric heavy chain gene was subcloned into

the mammalian expression vector pcDNAl. This vector contains the following DNA elements: the cytomegalovirus (CMV) immediate early promoter and enhancer driving transcription of the CD4-gamma2 chimeric heavy chain gene; an SV40 polyadenylation sequence; and an SV40 origin of replication which allows replication of the plasmid to high copy number in CosM5 cells. The resulting CD4-gamma2 heavy chain mammalian expression vector (designated CD4-IgG2-pcDNAl) was transfected into CosM5 cells which were then radiolabelled with ^{35}S-methionine 48-72 hours post-transfection. The radiolabelled medium was analyzed by precipitation with Protein ~~A-sepharose~~ A-Sepharose® beads and SDS-PAGE followed by fluorography (Figure 6). Under reducing conditions, a protein migrating at a relative molecular mass (Mr) of approximately 47 kilodaltons is precipitated. When the precipitated material was run on SDS-PAGE under nonreducing conditions, a protein migrating at an Mr of approximately 94 kilodaltons is observed, indicating that the CD4-gamma2 chimeric heavy chains assemble and are secreted as homodimers. In addition, these results demonstrate that the secreted CD4-gamma2 chimeric heavy chain homodimers contain an intact immunoglobulin Fc domain since they bind Protein A. Further characterization by Western blot analysis of the proteins secreted into the medium 48-72 hours post-transfection was performed using a rabbit polyclonal antiserum raised against purified soluble human CD4. Similar to the results obtained by precipitation, when the medium was run on SDS-PAGE under reducing conditions, followed by Western transfer to nitrocellulose, the major immunoreactive protein migrates at an Mr of approximately 47 kilodaltons. Under nonreducing conditions, the major immunoreactive protein migrates at an Mr of approximately 94 kilodaltons. Taken together, these results demonstrate that the CD4-gamma2 chimeric heavy chain is produced and secreted as a homodimer of the

predicted molecular weight.

The above results demonstrate that the Fc portion of CD4-gamma2 chimeric heavy chain homodimer, encoded by the constant regions of the gamma2 heavy chain gene, binds Protein A and is therefore functionally active. In order to determine if the CD4 portion is functionally intact, CD4-gamma2 chimeric heavy chain homodimers were assayed for their ability to bind to the HIV exterior envelope glycoprotein, gp120 (Figure 7). Unlabelled medium from CosM5 cells transfected withCD4-IgG2-pcDNA1 DNA was incubated with ^{35}S-methionine-labelled gp120. CD4-gamma2 chimeric heavy chain homodimer/gp120 complexes were precipitated by incubation with Protein ~~A-sepharose~~ A-Sepharose® beads, and the precipitates were analyzed by SDS-PAGE under reducing conditions followed by fluorography. These results demonstrate that the CD4-gamma2 chimeric heavy chain homodimer efficiently recognizes HIV gp120 and binds with high affinity. These observations, taken together with the results described in the above paragraph, demonstrate that CD4-gamma2 chimeric heavy chain homodimer contains functionally active regions of both CD4 and gamma2 heavy chain.

In order to stably produce large quantities of the CD4-gamma2 chimeric heavy chain homodimers, the CD4-IgG2-pcDNA1 vector was cotransfected with the plasmid p410 (encoding the enzyme dihydrofolate reductase (dhfr)) into dhfr-Chinese Hamster Ovary(CHO) cells. Approximately two weeks post-transfection, individual clones growing in nucleoside free alpha MEM and 10% dialyzed fetal calf serum (and therefore dhfr+) were isolated and analyzed for co-expression of CD4-gamma2 chimeric heavy chain homodimers by precipitation and ELISA. The highest producing cell lines were identified and subjected to stepwise increasing

concentrations of methotrexate which selects for amplification of the newly introduced DNA sequences. A CHO cell line expressing 10 micrograms/milliliter of CD4-gamma2 chimeric heavy chain homodimer was used for stable, constitutive production in roller bottles. The cells were grown to confluence in alpha MEM containing 10% IgG-free fetal calf serum. The cells were then fed every other day and two day old conditioned medium was used for purification of the CD4-gamma2 chimeric heavy chain homodimer. Conditioned medium was diluted 1:1 with phosphate-buffered saline (PBS) and applied to a 5ml column of Protein ~~A-sepharose~~ A-Sepharose® fast flow (Pharmacia) at a flow rate of 60 milliliters/hour. The column was then washed with 10 column volumes of PBS and the bound material was eluted with 100 mM glycine pH 3.5. The eluted material was collected directly into 50µl of 1M Tris.HC1 pH 8.0 to neutralize the eluant. Fractions having an OD(280) of greater than 0.1 were analyzed by SDS-PAGE followed by silver staining or Western blot analysis, and the peak fractions were pooled. A single band was specifically eluted from the Protein ~~A-sepharose~~ A-Sepharose® column with an Mr corresponding to the CD4-gamma2 chimeric heavy chain homodimer (Figure 8). Western blot analysis confirms that the eluted protein is immunoreactive with polyclonal antiserum raised against soluble human CD4. In addition, the purified protein retains the ability to bind with high affinity to ^{35}S-methionine-labelled gp120. These results demonstrate the stable, high-level production of CD4-gamma2 chimeric heavy chain homodimers in mammalian cells, and the purification of CD4-gamma2 chimeric heavy chain homodimer which retains biological function.

Please replace the paragraph beginning on page 52, lines 5-30 with the following amended paragraph:

Further purification of CD4-gamma2 heavy chain homodimer was achieved using ion-exchange chromatography. The peak fraction from the protein ~~A-sepharose~~ A-Sepharose® column was applied to a 10ml ~~S-sepharose~~ S-Sepharose® fast flow column preequilibrated with 50mM BES pH 7.0, at a flow rate of 120ml/hr. After application of the sample, the column was extensively washed with 50mM BES pH 7.0 with increasing salt concentration (see materials and methods). CD4-gamma2 heavy chain homodimer was specifically eluted from the column in 50mM BES pH 7.0 containing 500mM NaCl. Following the ion exchange chromatography, we unexpectedly found the peak fractions containing the CD4-gamma2 chimeric heavy chain homodimer was still impure. Therefore, the peak fractions from the ~~S-sepharose~~ S-Sepharose® column were pooled, concentrated and applied to a 120ml ~~Sephacryl~~ Sephacryl® S-300HR column preequilibrated with PBS and run at a flow rate of 8 ml per hour. The peak fractions of purified CD4-gamma2 heavy chain homodimer were analyzed by SDS-PAGE and silver staining under non-reducing conditions, and the purified fractions were pooled and analyzed by SDS-PAGE followed by silver staining under non-reducing conditions (Figure 11, lane 1), or reducing conditions (Figure 11, lane 2). When the purified CD4-gamma2 chimeric heavy chain homodimer was run on SDS-PAGE under reducing conditions, a doublet was observed which appeared to be due to differences in glycosylation of the CD4-gamma2 chimeric heavy chain homodimer (data not shown).

Please replace the paragraph beginning on page 53, line 9 to page 54, line 30 with the following amended paragraph:

Both the CD4-IgG2 chimeric heavy chain and the CD4-kappa chimeric light chain genes were subcloned into the mammalian expression vectors pRcCMV or pPPI-2. Both vectors contain the cytomegalovirus immediate early promoter and

enhancer driving transcription of the chimeric genes. In the vector pRcCMV, a second transcriptional cassette which contains the RSV promoter and enhancer is used to direct the transcription of the neomycin resistance gene. In pPPI-2, a second transcriptional cassette which contains the β-globin promoter directs the transcription of the dhfr gene (see supra). In order to stably produce large quantities of the CD4-IgG2 chimeric heterotetramer, the CD4-IgG2 chimeric heavy chain expression vector and the CD4-kappa chimeric light chain expression vector were transfected simultaneously (typically the CD4-IgG2 chimeric heavy chain gene cloned in pRcCMV was used, and CD4-kappa chimeric light chain gene cloned in pPPI-2 was used in a ratio of 1:1). Approximately two weeks post-transfection, individual clones growing in nucleoside-free alpha MEM containing 1 mg/ml G418 and 10% dialyzed fetal calf serum were isolated and analyzed for co-expression of both CD4-IgG2 chimeric heavy chains and CD4-kappa chimeric light chains by immunoprecipitation and ELISA. Figure 12 demonstrates one clone which was selected and analyzed for the expression of both CD4-IgG2 chimeric heavy chains and CD4-kappa chimeric light chains. The CHO cell line or the untransfected parental CHO cell line ~~were~~ was radiolabelled with ^{35}S-methionine and ^{35}S-cysteine for 16 hours. The radiolabelled medium was analyzed by precipitation with Protein ~~A-sepharose~~ A-Sepharose® beads and SDS-PAGE under non-reducing conditions followed by fluorography (Figure 12A). Under non-reducing conditions 2 proteins migrating at relative molecular masses of approximately 140 kilodaltons and 210 kilodaltons are precipitated. When the precipitated material was run on SDS-PAGE under non-reducing conditions, 2 proteins migrating at relative molecular masses of 69 kilodaltons and 35 kilodaltons were observed, which are consistent with the relative predicted molecular masses of the CD4-IgG2 chimeric heavy chains, and CD4-kappa chimeric

light chains respectively (data now shown). Further characterization has shown that the protein migrating at 210 kilodaltons on SDS-PAGE under non-reducing conditions contains both CD4-IgG2 chimeric heavy chains and CD4-kappa chimeric light chains which are covalently associated, while the protein migrating at 140 kilodaltons on SDS-PAGE under non-reducing conditions contains only CD4-IgG2 chimeric heavy chains (Figure 12B). These data are consistent with the predicted molecular weight of the 210 kilodalton protein having 2 CD4-IgG2 chimeric heavy chains and 2 CD4-kappa chimeric light chains, covalently associated to form a molecule with the structure H_2L_2 (H=heavy chain, [[L-]] L=light chain). Furthermore, the 140 kilodalton protein seen on SDS-PAGE under non-reducing conditions is consistent with the predicted molecular weight of a CD4-IgG2 chimeric homodimer having the structure H_2. Taken together, these results indicate that a CHO cell line which expresses both CD4-IgG2 chimeric heavy chains and CD4-kappa chimeric light chains is able to efficiently assemble and secrete CD4-IgG2 chimeric heterotetramers.